Research Note

Severe Pruning of Infected Grapevines Has Limited Efficacy for Managing Pierce's Disease

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Abstract: After the initial infection, bacterial plant pathogens often localize to specific tissues or within certain parts of their hosts. In such cases, it may be possible to clear the infection by removing the infected portion and retraining the plant from the base of the trunk. We tested the efficacy of severe pruning at clearing grapevine infections by *Xylella fastidiosa*, the causal agent of Pierce's disease. We surveyed vines in six Northern California vineyards and rated them on a scale of disease severity from 0 to 3. Next, we aggressively pruned vines by removing the trunk 10 cm above the graft union and we monitored their retrained canopies over time. Although 82% (284/346) of severely pruned vines appeared disease free the following season, the prevalence of symptoms in conventionally pruned control vines suggests that more than one-third (112/324) of vines would have recovered without severe pruning—at least those with less advanced symptoms. Moreover, for five of the six vineyards, the majority of severely pruned vines showed symptoms of Pierce's disease by the time vines were retrained, two seasons after pruning (as high as 81%, 86/106; 71% overall, 245/346). These results suggest that severe pruning does not clear *X. fastidiosa* infection from grapevines to an extent that would justify its adoption for disease management.

Key words: overwinter recovery, Pierce's disease, pruning, remedial surgery, roguing, sharpshooter, *Xylella fastidiosa*

There are multiple ways in which removal of infected host-plant tissue can be employed as an element of disease management. These include removal of reservoir hosts to limit pathogen spillover onto a focal host (Varela et al. 2001), roguing of infected focal hosts to limit secondary spread (Sisterson and Stenger 2013), and removal of localized infections within hosts to limit further infection or to retrain an unproductive plant (Sosnowski et al. 2011). Studies of bacterial pathogens in perennial crops have evaluated the utility of pruning as a disease management tool, with mixed results (Coletta-Filho et al. 2000, Lopes et al. 2007, Coletta-Filho and de Souza 2014). The removal of infected plant tissues is analogous to measures used for management of trunk diseases, often referred to as "remedial surgery," as an alternative to replacing infected plants (Sosnowski et al. 2011). In this study, we investigated whether severe pruning of *Xylella fastidiosa*-infected grapevines in commercial vineyards could clear vines of existing infections.

Pierce's disease (PD) is a lethal vector-borne disease of grapevines caused by the bacterium *X. fastidiosa* (Davis et al. 1978). After susceptible plants are inoculated by *X. fastidiosa*, pathogen populations multiply and move through the xylem network, leading to symptoms of reduced water flow (Newman et al. 2003), including leaf scorch, cluster desiccation, vine dieback, and eventually death. There is no cure for grapevines infected with this bacterium; current strategies for management of PD in California vineyards involve limiting pathogen spread to uninfected vines by controlling vector populations, disrupting transmission opportunities, and eliminating pathogen sources in the surrounding landscape (Varela et al. 2001, Almeida et al. 2005).

PD is notable for the numerous sources of variability in infection levels (i.e., bacterial titer, bacterial population, or density of colony forming units) and symptom severity in plants. *X. fastidiosa* infection levels vary among plant species (Hill and Purcell 1995), grapevine cultivars (Rashed et al. 2013), seasons (Hopkins 1981), and as a function of temperature (Feil and Purcell 2001). Like other bacterial plant pathogens (e.g., Saracco et al. 2006), *X. fastidiosa* is often irregularly distributed within individual hosts. For example, *X. fastidiosa* infection levels in grapevines may vary by more than 10-fold between grapevine petioles and stems (Krivanek and Walker 2005); in other hosts, infection levels may vary by more than 100-fold between basal and apical sections of shoots (Daugherty et al. 2010). This within-host heterogeneity may be epidemiologically significant if it affects pathogen

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acquisition efficiency (Daugherty et al. 2010). Moreover, if such variation is associated with protracted localized infection near inoculation points, such heterogeneity may facilitate other disease management tactics.

In addition to grapevines, other plant species that are susceptible to *X. fastidiosa* infection include citrus (*Citrus sinensis*) in South America (Coletta-Filho and de Souza 2014). Management of the resulting disease (citrus variegated chlorosis) in *C. sinensis* relies on clean nursery stock, vector control, and pruning infected plant tissue from established trees or roguing young plants (less than four-years-old) (Almeida et al. 2014, Coletta-Filho and de Souza 2014). The concept of pruning of infected plant material is based on the fact that, in established trees (>4 years), tissue with early symptoms of infection can be pruned ~1 m proximal to the most symptomatic basal leaf, effectively eliminating infections, as the remaining tissue is free of *X. fastidiosa* (Coletta-Filho et al. 2000). However, pruning is not adequate for young trees (<4 years) or for removing bacterial infections if any symptoms are present in fruit (summarized by Coletta-Filho and de Souza 2014).

X. fastidiosa multiplies and spreads through the xylem vessels, reaching the roots of perennial hosts such as citrus (He et al. 2000), peach (Aldrich et al. 1992), alfalfa (Daugherty et al. 2010), and blueberry (Holland et al. 2014). Nonetheless, under field conditions, chronic infection of grapevines is temperature and season dependent. In regions with freezing winter temperatures, infected plants can recover in winter, curing previously infected and symptomatic grapevines (Purcell 1977). Infections that occur during spring lead to chronic disease (i.e., the infection survives into subsequent years); however, infections that occur during late summer and fall may cause disease symptoms in the current year, but a high proportion of vines lack symptoms of *X. fastidiosa* infection in the following year (Feil et al. 2003). The biological mechanism behind this winter recovery has been studied but is not fully resolved. Nonetheless, models that incorporate low temperatures have substantial explanatory power in predicting rates of winter curing of *X. fastidiosa* infections in grapevine (Lieth et al. 2011). Infections that occur early in the season may have a longer period during which *X. fastidiosa* can colonize and reach high infection levels, which may increase the likelihood of the disease surviving over the winter. Following this rationale, if most late-season infections remain in the distal ends of shoots and have lower infection levels, removing the symptomatic portion of the vine might eliminate *X. fastidiosa*. In other words, the efficacy of pruning infected grapevine tissue could depend on both the time of year in which the plant was infected and on winter temperature.

A potential benefit of severe pruning versus replanting is that pruning leaves a mature rootstock in place, which is likely to support more vigorous regrowth compared to the developing rootstock of a young transplant (Varela et al. 2001). Recent attempts to increase vine productivity by planting vines with more well-developed root systems (Bettiga 2015) are based on this presumption. However, even if severe pruning can clear vines of infection, it removes a substantial portion of the aboveground biomass of the vine. Thus, a method for encouraging rapid regrowth of the scion after aggressive pruning is needed.

We studied the efficacy of pruning infected vines immediately above the rootstock graft union—the most aggressive pruning method—for clearing grapevines of infection by *X. fastidiosa*. We reasoned that if such severe pruning was ineffective at clearing vines of infection, less severe pruning would not be warranted; if severe pruning showed promise, less severe pruning could then be tested. We use the term "severe pruning" to refer to a special case of strategic pruning for disease management, analogous to the use of "remedial surgery" for trunk diseases (Sosnowski et al. 2011). To test the efficacy of clearing vines of *X. fastidiosa* infection, we followed the disease status of severely pruned versus conventionally pruned vines over multiple years, characterized the reliability of using visual symptoms of PD to diagnose infection, and compared two methods of restoring growth of severely pruned vines.

Materials and Methods

Study design. Pruning trials were established in Napa Valley, CA in commercial vineyards where symptoms of PD were evident in autumn of 1998. The vineyards used for these trials varied in vine age, cultivar, and initial disease prevalence (Table 1). All study vines were cordon-trained and spur-pruned. We mapped the portions of the six vineyards selected for study according to evaluation of vines for disease symptoms. The overall severity of PD symptoms for each vine was recorded as follows: 0 = no symptoms, apparently healthy; 1 = marginal leaf scorch on up to four scattered leaves total; 2 = foliar symptoms (marginal leaf scorch and/or petioles from which the blades have abscised) on one shoot or on fewer than half of the leaves on two shoots on one cordon, no extensive shoot dieback, and minimal (<25%) shriveling of fruit clusters; and 3 = foliar symptoms on two or more shoots (more than half of each) occurring in the canopy on both cordons; dead spurs possibly evident along with shriveled clusters.

To test the reliability of the visual diagnosis of PD, petiole samples were collected from the six vineyard plots when symptom severity was evaluated for vines in each symptom category; these samples were assayed using polymerase chain reaction (PCR) (Purcell et al. 1999). Petioles were collected from symptomatic leaves on 25, 56, and 30 vines in categories 1, 2, and 3, respectively.

Table 1 Summary of vineyard plots used in the study, including cultivar, vine age, and prevalence of Pierce's disease (%; number of symptomatic/total number of plants), based on visual symptoms, at the beginning of the study.

Plot	Cultivar	Age (yr)	Prevalence (%)
1	Cabernet Sauvignon	6	22.4 (321/1432)
2	Cabernet Sauvignon	4	2.0 (50/2498)
3	Cabernet franc	12	9.5 (109/1145)
4	Pinot noir	3	12.3 (204/1661)
5	Chardonnay	5	4.4 (74/1697)
6	Merlot	2	6.9 (81/1181)

Next, severe pruning was performed between October 1998 and February 1999 in the six vineyard plots by removing trunks of symptomatic vines ~10 cm above the graft union. Cuts were made with saws or loppers, depending upon the trunk diameter. During a vineyard survey, severe pruning was conducted on 50% of vines (every other vine) in each symptom category; the other 50% of vines served as conventionally pruned (nontreated) controls. Sample sizes for control and severely pruned vines in each disease category ranged between six and 62 vines depending on the plot, with at least 38 total vines per plot in each control or pruned treatment. In spring 1999, multiple shoots emerged from the remaining section of scion wood above the graft union on severely pruned vines. When one or more shoots were ~15 to 25 cm long, a single shoot was selected and tied to the stake to retrain a new trunk and cordons, and all other shoots were removed at this time. We evaluated the potential of severe pruning to clear vines of infection, by reinspecting both control and severely pruned vines in all six plots for the presence or absence of PD symptoms in autumn 1999 and 2000. In all plots, category 3 vines were inspected in a third year (autumn 2001); in plot 6, vines were inspected an additional two years (i.e., through autumn 2002).

Finally, in plot 6 we investigated chip-bud grafting (Alley 1979) as an alternate means of ensuring the development of a strong replacement shoot for retraining. To do this, 78 category 3 vines were selected for severe pruning, 39 of which were subsequently chip-bud grafted in May 1999. An experienced field grafter chip budded a dormant bud of *Vitis vinifera* cv. Merlot onto the rootstock below the original graft union, and the trunk and graft union were removed. The single shoot that emerged from this bud was trained up the stake and used to establish the new vine. The other 39 vines were severely pruned above the graft union and retrained in the same manner as vines in plots 1 to 5. Development of vines in plot 6, with and without chip-bud grafting, was evaluated in August 1999 using the following rating scale: 1) "no growth": bud failed to grow, no new shoot growth; 2) "weak": multiple weak shoots emerging with no strong leader; 3) "developing": selected shoot extending up the stake, not yet topped; and 4) "strong": new trunk established, topped, and laterals developing.

Statistical analysis. All analyses were conducted using R version 3.4.1 (R Development Core Team 2017). We used a generalized linear model (GLM) with binomial error to compare the relative frequency of *X. fastidiosa*-positive samples from vines in the different initial disease severity categories (Crawley 2009). Next, we analyzed the effectiveness of chip budding versus training of existing shoots as a means for restoring vines after severe pruning. This analysis used multinomial logistic regression that compared the frequency of four vine growth outcomes the following season: strong, developing, weak, or no growth. This main test was followed by pairwise Fisher exact tests of the frequency of each of the individual outcomes between chip budded-trained and trained vines (Crawley 2009).

We analyzed the effect of severe pruning on subsequent development of PD symptoms using two complementary analyses. First, we compared symptom return between severely pruned and control vines in the three symptom severity categories for two years after pruning. To appropriately account for repeated measurements made over time, our analysis consisted of a linear mixed-effects model with binomial error, a random effect of block, and fixed effects of treatment (i.e., severely pruned or control), year (one versus two years after pruning), and symptom severity category (1, 2, or 3). Next, we analyzed the rate at which PD reappeared in only severely pruned vines from category 3 in subsequent years using a survival analysis. Specifically, we used a Cox proportional hazards model (Crawley 2009) with a fixed effect of plot (i.e., six different combinations of cultivars and vine ages; Table 1).

Results and Discussion

PCR tests of samples from severely symptomatic (category 3) vines reliably confirmed *X. fastidiosa* infection; PCR was less reliable for categories 1 and 2. Binomial GLM showed a marginally significant relationship between disease severity rating and the fraction of vines that were *X. fastidiosa*-positive (AIC = 15.248, $\chi^2 = 5.895$, df = 2, $p = 0.0525$). A more parsimonious, preferred model (AIC = 13.263) that grouped disease severity categories 1 and 2 showed a significant relationship between disease severity and detection ($\chi^2 = 5.88$, df = 1, $p = 0.0153$). While detection was similar for the lowest two disease categories, averaging ~80% (Category 1 [mean ± SE] = 79.2 ± 8.3%; Category 2 [mean ± SE] = 80.4 ± 5.9%), the most severe disease category (3) had a significantly higher detection rate: only 1 of 30 putative diseased samples was negative (3.3 ± 3.3%).

Accurate and time- or cost-efficient methods of diagnosing infected plants are important elements of a disease management program, both with respect to roguing to reduce pathogen spread (e.g., Sisterson and Stenger 2013), and the efficacy of pruning to clear plants of infection (e.g., Coletta-Filho et al. 2000). Accurate diagnosis of PD in grapevines is complicated by quantitative and qualitative differences in symptoms among cultivars (Rashed et al. 2013) and other aspects of plant condition (e.g., water stress; Thorne et al. 2006). Our results suggest that a well-trained observer can accurately diagnose PD based on visual symptoms, particularly for advanced cases of the disease. The small number of false positives in disease category 1 and 2 vines may have been due to misdiagnosis of other biotic (i.e., trunk diseases) or abiotic factors (e.g., water stress; Thorne et al. 2006). Alternatively, false positives might indicate bacterial populations that are near the detection limit; conventional PCR has at least as low a detection threshold as other methods that rely on the presence of live bacterial cells (e.g., plate culturing; Hill and Purcell 1995). Regardless, although scouting based on visual symptoms clearly captured most cases of PD in the current study, some caution should be used when trying to diagnose early disease stages to ensure that vines are not needlessly removed.

There is no cure for grapevines once infected with *X. fastidiosa*, except for recovery that can occur in some overwintering vines (Feil et al. 2003). The virulent nature of *X. fastidiosa* in grapevines, and the corresponding high mortality

rate for early season infections, increases the potential value of any cultural practices that can cure vines of infection. Moreover, new vines replanted into established vineyards generally take longer to develop compared to vines planted in newly developed vineyards, potentially due to vine-to-vine competition for resources that limits growth of replacement vines. As a result, vines replanted in mature vineyards may never reach full productivity (R. Smith, personal observation, 2017). Thus, management practices that speed the regeneration of healthy, fully developed, and productive vines may reduce the economic loss caused by PD (Varela et al. 2001).

A multinomial logistic regression showed significant differences in the relative frequency of different grapevine growth outcomes between the two restoration methods ($\chi^2 = 26.692$, df = 1, $p < 0.0001$). Chip-budded vines showed significantly lower frequency of strong growth and significantly higher frequencies of vines with developing growth and, especially, of no growth (Table 2). Nearly 30% of chip-budded vines showed no growth in the following season, compared to 0% of vines on which established shoots were trained.

These results indicate that training newly produced shoots from the remaining section of the scion was more likely to result in positive regrowth outcomes. As a result, of the two methods we evaluated, training of shoots that emerge from the scion of a severely pruned trunk is recommended for restoring growth. However, it is important to note that the current study did not estimate the amount of time required for severely pruned vines to return to full productivity. Moreover, the study did not include mature (i.e., >15-year-old) vines, in which growth responses may differ from young vines. Additional studies may be needed to quantify vine yield, and perhaps fruit quality, in severely pruned vines over multiple seasons.

Table 2 Frequency of four different grapevine regrowth outcomes one season after severe pruning for two methods of restoring vine growth.

	Method[a]	
Vine growth outcome	**Trained after chip budding**	**Trained without chip budding**
Strong	15 a	33 b
Developing	12 d	4 e
Weak	1 g	2 g
None	11 i	0 j

[a]Different letters within rows denote significant differences between methods for a given vine growth outcome; n = 39 vines per treatment.

The usefulness of pruning for disease management depends on its ability to clear plants of pathogen infection (Coletta-Filho et al. 2000, Lopes et al. 2007, Coletta-Filho and de Souza 2014). A comparison of symptom prevalence among severely pruned and control vines from different disease severity categories showed significant effects of the number of years after pruning ($\chi^2 = 111.41$, df = 1, $p < 0.0001$), pruning treatment ($\chi^2 = 59.17$, df = 1, $p < 0.0001$), and initial disease symptom category ($\chi^2 = 214.01$, df = 1, $p < 0.0001$). The analysis also showed significant interactions between year and treatment ($\chi^2 = 41.48$, df = 1, $p < 0.0001$) and between treatment and symptom category ($\chi^2 = 24.11$, df = 1, $p < 0.0001$), a nonsignificant interaction between year and symptom category ($\chi^2 = 2.14$, df = 1, $p = 0.14347$), and a marginally significant three-way interaction ($\chi^2 = 2.96$, df = 1, $p = 0.0855$). Overall, more vines had symptoms in the second year compared to the first (44% after one year and 71% after two years), and there was a higher prevalence of returning symptom in vines from higher initial disease categories (27, 62, and 82% for symptom categories 1, 2, and 3, respectively). Severe pruning showed an apparent benefit to reducing symptoms of PD after the first year, but this effect weakened substantially by the second year, with no differences for category 1 or 3 vines, and a slightly lower disease prevalence for severely pruned category 2 vines (Figure 1). A survival analysis of severely pruned category 3 vines showed a significant difference in the rate of symptom return among

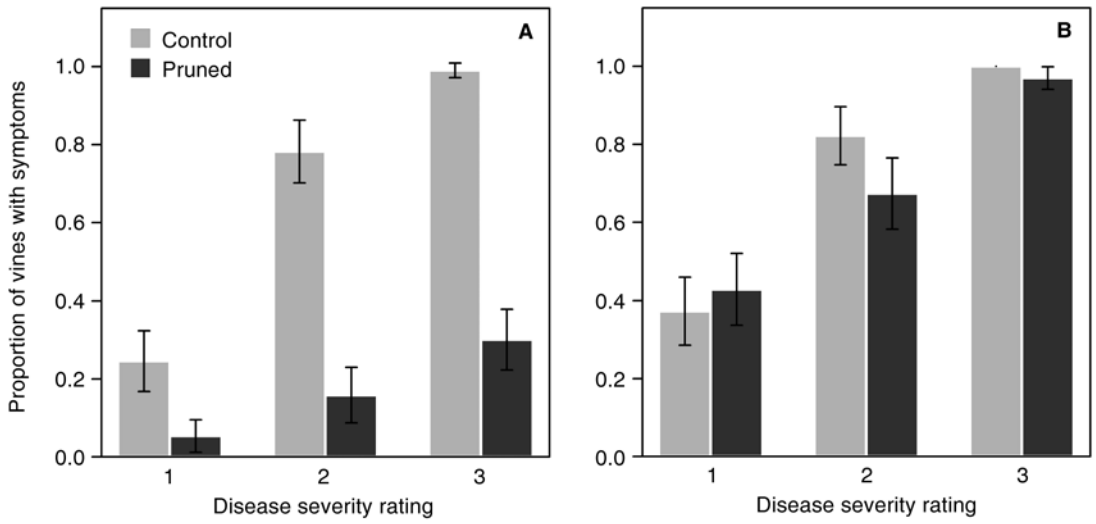

Figure 1 Return of Pierce's disease symptoms in severely pruned or control (conventionally pruned) vines from three disease-severity categories after **A**) one year or **B**) two years. Each column represents the average proportion of vines with symptoms, for groups of 101 to 133 vines spread among six vineyard blocks. Error bars denote 95% confidence intervals.

plots (Wald test = 96.8, $p < 0.0001$). All vines in plots 1 to 3 had symptoms by autumn 2000, two years after pruning (Figure 2). In plots 4 and 5, more than 80% of vines showed symptoms after three years. Only plot 6 showed markedly lower disease prevalence; in plot 6, ~70% (47/67) and 50% (33/67) of severely pruned category 3 vines showed no symptoms after two and four years, respectively, versus ~36% of control vines overall, after two years.

It is important to note that at the time of this study, disease pressure may not fully explain the return of symptoms in severely pruned vines. Surveys conducted during the first two years of the study throughout the entirety of the six research blocks showed that the prevalence of PD in control vines actually declined slightly from the first to the second year (mean ± SD change in % of vines: -4.5 ± 4.56%), but not due to an increase in replanting efforts or vine death (mean ± SD change in % of replanted, dead, or missing vines was 0.33 ± 2.17%), Rather, this decline in prevalence likely reflects overwinter recovery of mild cases of the disease (i.e., category 1 vines; Purcell 1974). Thus, the observed return of symptoms in most severely pruned vines does not appear to be explained by reinfection with *X. fastidiosa* after clearing of infection during the severe-pruning process.

Our results indicate that the apparent effectiveness of severe pruning depended on the initial disease severity, and the effectiveness weakened over time. This suggests at least two constraints exist regarding the general utility of pruning as a PD management tool. First, severe pruning does not appear to be useful for mild cases of PD, as many of those same vines would recover from the infection over the winter (Purcell 1974, 1977, Feil et al. 2003). Second, there appears to be little value in pruning severely diseased vines; the high frequency of symptom return within a few years indicates that even severe pruning does not clear most vines of *X. fastidiosa* infection. That leaves a statistically significant window with respect to intermediate severity cases, which may benefit from severe pruning. The apparent benefit for this category of diseased vines would stem from infections that are not so localized that they are highly susceptible to natural recovery over the winter, but also not fully systemic such that the infection has developed below the pruning point (e.g., Lopes et al. 2010). Reliable identification of this narrow class of diseased vines may require substantial experience with PD scouting, detailed record keeping, and an appreciation for variability in symptoms or infection dynamics based on grapevine cultivar (Rashed et al. 2013) and environmental conditions (Thorne et al. 2006, Lieth et al. 2011).

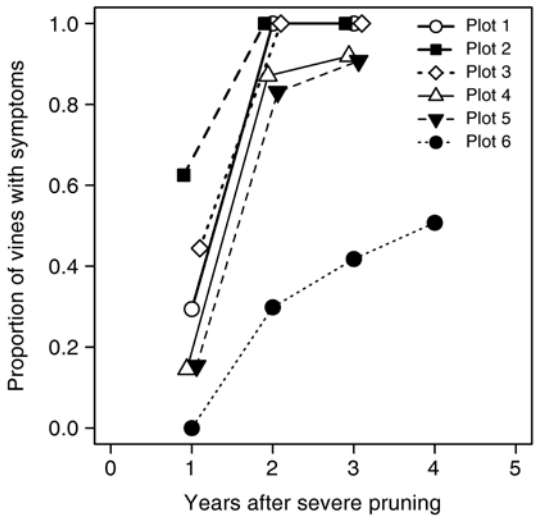

Figure 2 Return of Pierce's disease symptoms after severe pruning of vines in the most severe disease category, for the six vineyard plots. Some plot symbols offset slightly for clarity. Points represent the overall proportion of vines that showed symptoms for up to 67 replicate severely pruned vines per block.

Research in other bacterial plant pathosystems has evaluated the potential benefit of pruning (Coletta-Filho et al. 2000, Coletta-Filho and de Souza 2014) and whether pruning extent is related to its effectiveness at clearing hosts of infection (Lopes et al. 2007). A study of the citrus disease huanglongbing, associated with infection by *Candidatus* Liberibacter spp., evaluated two levels of pruning severity, neither of which showed promise as a disease management tool (Lopes et al. 2007). In this pathosystem, it is plausible that a very protracted incubation period (i.e., several months to years; Coletta-Filho et al. 2014) may undermine the effectiveness of pruning, because by the time the first symptoms are visible, the infection may have already moved throughout much of the tree. Collectively, our results are more similar to a study of citrus variegated chlorosis (Coletta-Filho et al. 2000). In this study, the presence of *X. fastidiosa* in plant tissues at different distances from symptomatic leaves was determined for varying levels of disease severity. *X. fastidiosa* was more widely distributed in trees with severe disease symptoms compared to those with early stage foliar symptoms. Although Coletta-Filho et al. (2000) did not test whether pruning at various distances proximal to symptomatic leaves would eliminate *X. fastidiosa* infections, the current recommendation is to prune citrus material if early symptoms are present, and to not prune plants with severe disease symptoms (Coletta-Filho and de Souza 2014). Citrus plant age is also an important consideration; Coletta-Filho and de Souza (2014) recommend that symptomatic citrus trees up to three-years-old be removed rather than pruned, whereas trees four-years-old or older should be pruned. We did not examine vine age as a factor in this study, but the biology of citrus and grape differ in terms of the overwinter recovery that can occur in grape (Feil et al. 2003) and the apparently slower movement of *X. fastidiosa* in citrus compared to grape. Anecdotally, the two most mature plots in our study showed the most rapid return of disease, and the youngest plot showed the slowest return. More studies of the effect of vine age are needed before concluding that interactive effects of plant age and pruning differ between the PD and citrus variegated chlorosis pathosystems.

Conclusions

Severe pruning of *X. fastidiosa*-infected grapevines appears to temporarily reset PD severity for up to a few years. Severe pruning generally does not clear the infection; PD symptoms eventually return to the majority of vines. Although certain

contexts (i.e., specific combinations of cultivars, climate, and disease stages) may incrementally improve the long-lasting effect of severe pruning, the removal of substantial portions of the plant associated with the pruning treatment is likely to have detrimental, multi-season impacts on vine regrowth, yield, and berry quality. As a result, severe pruning should not be viewed as a broadly applicable PD management strategy.

Literature Cited

Aldrich JH, Gould AB and Martin FG. 1992. Distribution of *Xylella fastidiosa* within roots of peach. Plant Dis 76:885-888.

Alley CJ. 1979. Chip-budding of mature grapevines. Calif Agric 33:14-16.

Almeida RPP, Blua MJ, Lopes JRS and Purcell AH. 2005. Vector transmission of *Xylella fastidiosa*: Applying fundamental knowledge to generate disease management strategies. Ann Entomol Soc Am 98:775-786.

Almeida RPP, Coletta-Filho HD and Lopes JRS. 2014. *Xylella fastidiosa*. *In* Manual of Security: Sensitive Microbes and Toxins. Liu D (ed.), pp. 841-850. CRC Press, Boca Raton, FL.

Bettiga L. 2015. Comparison of benchgraft and training strategies on the development and productivity of Chardonnay grapevines. *In* 66th ASEV National Conference, pp. 58-59. Portland, OR.

Coletta-Filho HD and de Souza AA. 2014. Avanços no conhecimento sobre a clorose variegada dos citros: Uma abordagem sobre os diferentes components do patossistema. Citrus Res Technol 35:19-33.

Coletta-Filho HD, Carlos EF, Targon MLPN, Cristofani M, Souza AA and Machado MA. 2000. Distribution of *Xylella fastidiosa* within sweet orange trees: Influence of age and level of symptom expression of citrus variegated chlorosis. *In* Proceedings of the 14th Conference of the International Organization of Citrus Virologists. da Graça et al. (eds.), pp. 243-248. Riverside, CA.

Coletta-Filho HD, Daugherty MP, Ferreira C and Lopes JRS. 2014. Temporal progression of '*Candidatus* Liberibacter asiaticus' infection in citrus and acquisition efficiency by *Diaphorina citri*. Phytopathology 104:416-421.

Crawley MJ. 2009. The R Book. Wiley and Sons, Inc., Chichester, England.

Daugherty MP, Lopes J and Almeida RPP. 2010. Vector within-host feeding preference mediates transmission of a heterogeneously distributed pathogen. Ecol Entomol 35:360-366.

Davis MJ, Purcell AH and Thomson SV. 1978. Pierce's disease of grapevines: Isolation of the causal bacterium. Science 199:75-77.

Feil H and Purcell AH. 2001. Temperature-dependent growth and survival of *Xylella fastidiosa* in vitro and in potted grapevines. Plant Dis 85:1230-1234.

Feil H, Feil WS and Purcell AH. 2003. Effects of date of inoculation on the within-plant movement of *Xylella fastidiosa* and persistence of Pierce's disease within field grapevines. Phytopathology 93:244-251.

He CX, Li WB, Ayres AJ, Hartung JS, Miranda VS and Teixeira DC. 2000. Distribution of *Xylella fastidiosa* in citrus rootstocks and transmission of citrus variegated chlorosis between sweet orange plants through natural root grafts. Plant Dis 84:622-626.

Hill BL and Purcell AH. 1995. Multiplication and movement of *Xylella fastidiosa* within grapevine and four other plants. Phytopathology 85:1368-1372.

Holland RM, Christiano RSC, Gamliel-Atinsky E and Sherm H. 2014. Distribution *of Xylella fastidiosa* in blueberry stem and root sections in relation to disease severity in the field. Plant Dis 98:443-447.

Hopkins DL. 1981. Seasonal concentration of the Pierce's disease bacterium in grapevine stems, petioles, and leaf veins. Phytopathology 71:415-418.

Krivanek AF and Walker MA. 2005. *Vitis* resistance to Pierce's disease is characterized by differential *Xylella fastidiosa* populations in stems and leaves. Phytopathology 95:44-52.

Lieth JH, Meyer MM, Yeo KH and Kirkpatrick BC. 2011. Modeling cold curing of Pierce's disease in *Vitis vinifera* 'Pinot Noir' and 'Cabernet Sauvignon' grapevines in California. Phytopathology 101:1492-1500.

Lopes JRS, Daugherty MP and Almeida RPP. 2010. Strain origin drives virulence and persistence of *Xylella fastidiosa* in alfalfa. Plant Pathol 59:963-971.

Lopes SA, Frare GF, Yamamoto PT, Ayres AJ and Barbosa JC. 2007. Ineffectiveness of pruning to control citrus huanglongbing caused by *Candidatus* Liberibacter americanus. Eur J Plant Pathol 119:463-468.

Newman KL, Almeida RPP, Purcell AH and Lindow SE. 2003. Use of a green fluorescent strain for analysis of *Xylella fastidiosa* colonization of *Vitis vinifera*. Appl Environ Microbiol 69:7319-7327.

Purcell AH. 1974. Spatial patterns of Pierce's disease in the Napa Valley. Am J Enol Vitic 25:162-167.

Purcell AH. 1977. Cold therapy of Pierce's disease of grapevines. Plant Dis Rep 61:514-518.

Purcell AH, Saunders SR, Norberg E and McBride JR. 1999. Reductions of Pierce's disease vector activity by management of riparian woodlands. Phytopathology 89:S62.

R Development Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rashed A, Kwan J, Baraff B, Ling D, Daugherty MP, Kiliny N and Almeida RPP. 2013. Relative susceptibility of *Vitis vinifera* cultivars to vector-borne *Xylella fastidiosa* through time. PLoS ONE 8:e55326.

Saracco P, Bosco D, Veratti F and Marzachì C. 2006. Quantification over time of chrysanthemum yellows phytoplasma (16Sr-I) in leaves and roots of the host plant *Chrysanthemum carinatum* (Schousboe) following inoculation with its insect vector. Physiol Mol Plant P 67:212-219.

Sisterson MS and Stenger DC. 2013. Roguing with replacement in perennial crops: Conditions for successful disease management. Phytopathology 103:117-128.

Sosnowski MR, Wicks TJ and Scott ES. 2011. Control of Eutypa dieback in grapevines using remedial surgery. Phytopathol Mediterr 50:S277-S284.

Thorne ET, Stevenson JF, Rost TL, Labavitch JM and Matthews MA. 2006. Pierce's disease symptoms: Comparison with symptoms of water deficit and the impact of water deficits. Am J Enol Vitic 57:1-11.

Varela LG, Smith RJ and Philips PA. 2001. Pierce's disease. Publication 21600. University of California Agriculture and Natural Resources, Oakland, CA.